CHAUDHARY RANBIR SINGH UNIVERSITY
ESTABLISHED BY GOVT. OF HARYANA LEGISLATURE ACT NO.28 OF 2014
(RECOGNIZED UNDER SECTION 12(B) & 2(F) OF UGC ACT, 1956)

Practical Marks (2022 - 2023)

College :- (1616)Govt. PG College, Jind

Degree :- B.Sc-(VIth Sem)

Subject :- BIO TECHNOLOGY

Paper :- (BT06(III)) Project Work (In House) (Max Marks - 50)

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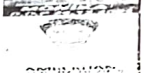
11	7900202	12006803005 1	AMIR KHAN	ALIMU DIN	KRISHNA DEVI	30		Amirkhan
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Online marks submission Date

Hard copy Submission Date .....

College /invigilator Signature .....

04.5.23

CHAUDHARY RANBIR SINGH UNIVERSITY
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09.5.23

Project Report

on

Micro propagation of *Tagetes erecta*

Submitted to

Chaudhary Ranbir Singh University, Jind

For partial fulfillment of requirements

of the degree of

BACHELORS OF SCIENCE

ADVISOR

Dr. Virender Kumar

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CHAUDHARY RANBIR SINGH UNIVERSITY

JIND (HARYANA)

Session: 2020-23

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Everyone aspires to achieve an enjoyable feeling and power through different activities and efforts. This one month training is a small Endeavour to obtain power in the field of education.

As we cannot separate moon from moonlight and stars and flowers from fragrance in the same way on the path of life, the forwarding steps strengthened by those who gave stony foundation, affection, guidance cannot be forgotten.

I am grateful to the current principal (Mrs. Veena Behl), previous principals and HOD Biotechnology Department for maintaining the facilities in the department, which helped us to complete this project in time.

It is my profound privilege to express my indebtedness and deep sense of gratitude towards my esteemed advisor Dr. Virender Kumar and Dr. Poonam, Lecturers, Govt. college, Jind for his/her keen interest, constructive criticism, valuable suggestions and above all highly inspiring, guidance which shaped the work into present.

Last but not least my thanks to all my family members for inspiring me to complete the study.

Above all, I am grateful to the Almightily God continuously serving a light house for blessing me the patience, courage and confidence to go ahead with task and make a success.

SHIKSHA

CERTIFICATE

This is to certify that **SHIKSHA** student of B.Sc. Biotechnology has completed one month Project entitled "**MICROPROPAGATION OF *Tagetes erecta***" which is being submitted to the Govt. College, Jind.

To the best of my knowledge the work has not been submitted in part or in full to any other university or institute for the award of any degree.

6/05/23

Advisor

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CHAPTER 1

INTRODUCTION

(Fig.1) *Tagetes erecta*

CHAPTER 1

INTRODUCTION

Natural plant products have been used throughout human history for various purposes .Many of these natural products have biological activity that can involve in drug discovery and drug design. The Indian system of medicine known as "Ayurveda" uses mainly plant based drugs or formulations to treat various ailments, including cancer. Herbal drugs have great growth potential in global market. Research work on the chemistry of natural products, pharmacognosy, pharmaceutics, pharmacology and clinical therapeutics have been carried out by herbal drugs and most of the leading pharmaceutical corporations have revised their strategies in favour of natural products. Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. The therapeutic value of *Tagetes erecta (Fig. 1)*, commonly known as Marigold, has been recognized in different system of traditional medicine for the treatment of different human aliments (Dixit et al.2013). *Tagetes* is a genus (family compositae/Asteraceae) containing about 50 species of annual or perennial herbaceous plant. The plant *Tagetes erecta* L is locally known as Genda Phool. Marigold is a spice native to India. Historically, marigold has been used all over in India, China and Indonesia as a spice and medical agent. Marigold is a spice that enhances the flavor of foods and is the base of most Indian curries. Marigold is used in curries goes back more then 5000 years. It is stout, branching herb native to Mexico and other warmer parts of America and neutralized elsewhere in the tropic and subtropics including India and Bangladesh. These are rapid growing annual flowering plants in height ranging from dwarf of 6-8inch, to medium and taller and erect growing plants with height from 10 into 3ft, bearing large pompom like double flower up to 5 in across and has a shorter flowering period from midsummer to frost . IT is very popular as a garden plant and yields a strongly aromatic essential oil, which is mainly used for compounding of high grade perfumes. Different parts of this plant including flowers are used in folk medicines to cure various diseases. Leaves are used as antiseptics and in kidney troubles, muscular pain, piles and applied to boils and carbuncles.

Objective:

- Micro propagation of *Tagetes erecta(Fig.2)* using nodal portion.

(Fig.2) *Tagetes erecta*

CHAPTER 2

Literature Review:

CHAPTER 2

REVIEW OF LITERATURE

There have been several studies conducted on the micro propagation of marigold, and their findings suggest that micro propagation is a viable method for the mass production of marigold plants. Some of the notable studies are

•In vitro propagation of French marigold (*Tagetes erecta L.*) through axillary shoot proliferation by Saha et al. (2008) - The study showed that nodal explants of marigold were capable of producing multiple shoots when cultured on a medium containing a combination of plant growth regulators. The highest number of shoots (7.2) was obtained with the medium containing 0.5 mg/L of both benzyl adenine (BA) and indole-3-butyric acid (IBA).

•In vitro propagation of African marigold (*Tagetes erecta* L.) through shoot tip culture by Hussain et al. (2010) - The study found that shoot tips were the best explants for the micropropagation of African marigold. The medium containing 2.0 mg/L of BA and 0.1 mg/L of IBA produced the highest number of shoots (8.9) per explant after 30 days of culture.

"In vitro propagation of marigold (Tagetes spp.) through axillary shoot proliferation" by Sharma et al. (2013) - The study reported that nodal explants of marigold were able to produce multiple shoots when cultured on a medium containing 0.5 mg/L of BA and 0.1 mg/L of IBA. The highest number of shoots (8.8) was obtained after 28 days of culture

•Effect of cytokinins on in vitro regeneration of marigold (*Tagetes spp.*) by Kumar et al. (2014) - The study found that the combination of BA and kinetin (Kin) was the most effective for the regeneration of marigold shoots. The medium containing 1.0 mg/L of BA and 0.5 mg/L of Kin produced the highest number of shoots (8.6) per explant after 25 days of culture.

...as Marigold flower. Flowers are edible and also used as coloring ...d from the flower can be used as a saffron substitute ...se of the flower as an edible dye.

Marigold is a hardy annual herb native to Southern Europe which can also be found growing in most temperate regions of the world. They grow up to 50-80cm in height, the leaves mid-green, lanceolate and between 5-17cm in length. The leaves and stem are covered with small hairs, the edges of leaves can be sparsely toothed or wavy. The plant grows to a height of one to five feet and is cultivated extensively in Asia, India, China and other countries with a tropical climate. The marigold plant needs temperature between 20 degree Celsius and 30 degree Celsius and a considerable amount of annual winter and rainfall to thrive.

The world production of marigold stand at around 600000 tones, of which India has a share of approx. 75-80% .India consumes about 80% of its own production. Indian marigold is considered the best in the world. India exports marigold flower to discerning countries like Japan, Sri Lanka, Iran, North African countries, US, and UK . The production of marigold is concentrated in the southern part of the countries, mainly in the peninsula area. Andhra Pradesh, Utter Pradesh is the leading marigold producing state in India followed by Tamil Nadu, Andhra Pradesh also has the highest area under marigold cultivation. Maximum area under marigold in Andhra Pradesh followed by Maharashtra, Tamil Nadu, Orissa, Karnataka, U.P and Kerala. The genus *Tagetes* contains many taxes which are economically important as food, condiment and as coloring medicinal and ornamental materials. The highest diversity is concentrated in India and Thailand, with at least 50 species in each area, followed by other countries.

Tagetes erecta is the main species of commerce and distributed its flower in India china and also in Sri Lanka, Indonesia, Jamaica and Peru, a city in the south Indian state of Tamil Nadu is India's largest producer and the most important trading center for marigold (Majumder et al, 2014) .The optimum time for cultivation and harvesting of marigold is given in table Given below

SEASON	SOWING TIME	TRANSPLANTING TIME	HARVESTING TIME
RAINY	JUNE-JULY	JULY-AUGUST	SEPTEMBER-OCTOBER
SUMMER	JANUARY	FEBRUARY	MARCH-APRIL
WINTER	SEPTEMBER-OCTOBER	OCTOBER-NOVEMBER	NOVEMBER-DECEMBER

TABLE: The optimum time for cultivation and harvesting of marigold

2.1 CHEMICAL CONSTITUENTS

Photochemical studies of its different parts have resulted in the isolation of various chemical constituents such as Thiophenes, carotenoids and triterpenoids. The plant *T.erecta* has been shown to contain quercetagetin, a glucoside of quercetagetin, phenolics, syringic acid, methyl -3, 5-dihydroxy-4methoxy benzoate, auercetin, vinyl and ethyl gallate. Lutein is an oxycarotenoid, or xanthophyll, containing 2 cyclic end groups (one beta and one alpha-ionone ring) and the basic C-40 isoprenoid structure common to all carotenoids. It is one of the major constituent and the main pigment of *Tagetes erecta* (Dixit et al, 2013).

The flower consists of carotenoids containing of lutein, zeaxanthin, neoxanthin plus violaxnthin, beta-carotene, lycopene, alpha-cryptoxanthin, phytoene and phytofluene. Li-Wei (2011) report the results of a thorough phytochemical study on 22 compounds from the flower of T.erecta by isolation of various fractions of the ethanol extract by silica gel column chromatography. They were beta-sitosterol, duacosterol, 7beta-hydroxysitosterol, erythrodiol-3-palmiate, lupeol, erythrodiol, etc. The chemical structure of lutein, quercetagetin and syringic acid are given below.

2.2 PHARMACOLOGICAL ACTIVITES

Tagetes erecta shows diverse pharmacological activities which are shown below in the following heads.

1. **Antibacterial activity**

The antibacterial activity of different solvents of *Tagetes erecta* flowers against Alcaligens faecalis, Bacillus cereus, Campylobacter coli, Escherichia coli, Klebsiella, pneumonia, pseudomonas, aeruginosa, Proteus vulgaris, Streptococcus pvagenes. The flavonoid possesses antibacterial activity against all the tested strains and shows a maximum zone of inhibition for Klebsiella pneumonia (29.50mm) .Flavonoid-patulitrin is one of the potential element for its antibacterial activity. The flower parts showed maximum inhibitory action against Neisseria gonorrhea strain (Patrick et al., 2011) .

2. Antinociceptive and anti-inflammatory activity

Antinociceptive and anti-inflammatory activity of chloroform, methanol and ether fraction of chloroform, methanol and ether fraction of Tagetes erecta reported by using acetic acid induced writhing in mice and carrageenan-induced paw oedema in the rat (Shinde et al, 2009). Antinoceptive and anti-inflammatory activity of hydro alcoholic extract of leaves of Tagetes erecta reported by using acetic acid-induced writhing and hot plate in mice and carrageenan – induced paw oedema in rat (Chatteriee et al, 2009).

3. **Anti-oxidant activity**

The ethanolic extract of Tagetes erecta flowers showed anti-oxidant activity by three different assays like DPPH, reducing power and superoxide radical scavenging activity at different concentration were used. In all the three assays Tagetes erecta showed better reducing power than the standard (i.e. ascorbic acid), superoxide anion scavenging activity and DPPH antioxidant activity showed less than standard (Chivde et al, 2011). The essential oil of flower of Tagetes erecta produced antioxidant activity by using DPPH, Thiocyanate, beta-carotene bleaching, free radical scavenging activity and oxidation of deoxyribose assay (Maratha et al, 2006).

4. Mosquitocidal activity

Mosquitocidal effect of ethanolic extract of flower of Tagetes erecta and its chloroform and petroleum ether soluble fraction against the larvae of Culex quinquefasciatus have been investigated. The larvicidal effect of ethanol extract and their solvent fraction were determined by the standard procedure of WHO against different instars of C. quinquefasciatus (Nikkon et al, 2011).

5. Anti-fungal activity

Fungi toxic activity of the essential oil of leaf Tagetes erecta exhibited complete inhibition of the growth Pythium aphanidermatum, the damping – off pathogen, at a concentration of 2000 ppm (Kishorae et al, 2006).

6. Hepatoprotective activity

Ethyl acetate fraction of Tagetes erecta at the dose of 400 mg/kg orally significantly decreased the elevated serum ALT, AST, ALP and level of bilirubin almost to the normal level compared to CCL4-intoxicated group. Histological changes in the liver of rat treated with 400mg/kg of the extract and CCL4 showed a significant recovery except for cytoplasmic vascular degeneration around portal tract, mild inflammation and foci of lobular inflammation (Giri et al, 2011).

7. Anti-cancer activity

Marigold has long been used as a medicinal herb for a number of therapeutic activities. The cytotoxic activity of ethanol and ethyl acetate extract of marigold flowers and their inhibitory *effect* on elastase and tyrosinase enzyme were investigated. An assay was performed to measure the cytotoxicity of these two extract on the H460 lung cancer and the $CaCO_2$ colon cancer cell lines (Vallisuta et al, 2014).

8. Anti-epileptic activity

The ethanolic extract Tagetes erecta was evaluated using the in vivo models such as pentobarbitone induced sleeping time, MES and PTZ induced convulsion, potentiation of PTZ induced convulsion, spontaneous locomotor activity, forced swim test and learned help-

lessness test model. The ethanolic extract Tagetes erecta showed antiepileptic activity. The finding suggested that ethanolic extract may reduce the seizure threshold in epileptic patient, chances of seizure precipitation is more, the usage in epilepsy is cautious (Shetty et al, 2009).

9. Other activity

In traditional medicine, marigold petals have been made into ointments, extract and infusions and used for a variety of ailments, including:

a. Fever
b. Jaundice
c. Stomach ulcer
d. Liver problem
e. To stop bleeding
f. Burns and wound
g. Conjunctivitis (pink eye)

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

Source of explants

The explants are collected from ground of Govt.college, Jind

3.1. Micro propagation

Experiments were planned to grow the plant by nodal portion. These experiments were carried out in Govt.college, Jind.

3.2. Different instruments

a. Laminar air flow:

Laminar air flow (fig.3) is an enclosed bench designed to prevent contaminations like biological particles or any particles sensitive device. This closed cabinet is usually made up of stainless steel without any gap or joints where spores might collect. Air is drawn through a HEPA filter and blown in a very smooth, laminar flow towards the user. Due to the direction of air flow, the sample is protected from the user but the user is not protected from the sample. The cabinet is usually made of stainless steel with no gaps or joints where spores might collect. Such hoods exist in both horizontal and vertical configurations, and there are many different types of cabinets with a variety of airflow patterns and acceptable uses.

Laminar flow cabinets may have a UV-C germicidal lamp to sterilize the interior and contents before usage to prevent contamination of the experiment. Germicidal lamps are usually kept on for fifteen minutes to sterilize the interior before the cabinet is used. The light must be switched off when the cabinet is being used, to limit exposure to skin and eyes as stray ultraviolet light emissions can cause cancer and cataracts.

(Fig.3) Laminar air flow

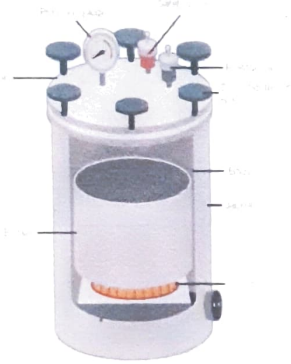
b. Hot air oven:

Hot air ovens (Fig.4) are electrical devices which use dry heat to sterilize. They were originally developed by Louis Pasteur. Generally, they use a thermostat to control the temperature. Their double walled insulation keeps the heat in and conserves energy, the inner layer being a poor conductor and outer layer being metallic. There is also an air filled space in between to aid insulation. An air circulating fan helps in uniform distribution of the heat. These are fitted with the adjustable wire mesh plated trays or aluminum trays and may have an on/off rocker switch, as well as indicators and controls for temperature and holding time. The capacities of these ovens vary. Power supply needs vary from country to country, depending on the voltage and frequency (hertz) used. Temperature sensitive tapes or biological indicators using bacterial spores can be used as controls, to test for the efficacy of the device during use.

(Fig.4) Hot air oven

c. Autoclave:

Autoclave (Fig.5) is also known as **steam sterilizers**, and is typically used for healthcare or industrial applications. An autoclave is a machine that uses steam under pressure to kill harmful bacteria, viruses, fungi, and spores on items that are placed inside a pressure vessel. The items are heated to an appropriate sterilization temperature for a given amount of time. The moisture in the steam efficiently transfers heat to the items to destroy the protein structure of the bacteria and spores. This principle is employed in an autoclave where the water boils at 121°C at the pressure of 15 psi or 775 mm of Hg.



(Fig.5) Autoclave

d. Hotplate:

A hotplate (Fig.6) is a portable self-contained tabletop small appliance cooktop that features one or more electric heating elements or gas burners. A hot plate can be used as a stand-alone appliance, but is often used as a substitute for one of the burners from an oven range or a kitchen stove. Hot plates are often used for food preparation, generally in locations where a full kitchen stove would not be convenient or practical. They can also be used as a heat source in laboratories. A hot plate can have a flat surface or round surface. Hot plates can be used for traveling or in areas without electricity.

(Fig.6) Hot plate

e. Different glassware:

Like measuring cylinder, beakers, conical flasks, petriplate, pipette (Fig.7) etc. are required for nutrient media.

(Fig.7) Different glassware

f. pH pen meter (Fig.8):

For tissue culture, the preferred pH of the media is between 5.8 and 6.0, which is a slightly acidic and neutral condition.

(Fig.8) pH pen meter

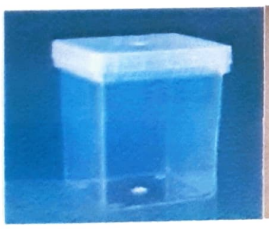
g. Weighing machine:

A weighing machine (Fig.9) is an instrument that is used to determine the weight or mass of an object. It is available in a wide range of sizes with multiple weighing capacities and is an essential tool in laboratories.

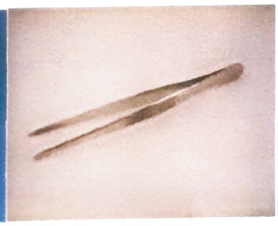
(Fig.9) Weighing machine

h. Different tools used:

Magenta box (fig.10), Forceps (fig.11), scalpel (fig.12), burner (fig.13) are used in plant tissue culture.



(Fig.10) Magenta box



(Fig.11) Forcep

(Fig.12) Scalpel

(Fig.13) Burner

3.3. Media

Murasshige and Skoog's medium (Murasshige and Skoog, 1962) is a land mark in plant tissue culture research and is the most frequently used medium for all types of tissue culture work. Based on its composition, other media were evolved to meet the diverse experimental and species specific requirement.

There are Linsmaier and Skoog (1965), B5 medium of Gamborg et. al. (1968), SH medium of Schenk and Hildebrandt (1972), Nitsch and Nitsch (1969) medium, and woody plant medium (WPM) of Lloyd and McCown, (1980).

The successful plant tissue culture depends upon the choice of nutrient medium. The cells of most plant species can be grown on completely defined media. All the media consist of mineral salts, a carbon source (generally sucrose), vitamins and growth regulators. The MS medium designed for tobacco is now used widely for various species, in callus and cell cultures.

Medium Composition:

The MS (Fig.14) medium for most plant tissue cultures is comprised of five groups of ingredients – inorganic nutrients, carbon source, vitamins, growth regulators and organic supplements.

(A) Inorganic Nutrients:

Inorganic nutrients consist of macro- and micro-elements as their salts. Usually nutrient media contain 25 mM each of nitrate and potassium. For regular culture and cell cultures, the combined nitrogen level (nitrate and ammonium nitrogen) may reach up to 60 mM.

Ammonium is essential for most cultures but in lower concentrations than that of nitrate nitrogen. A concentration of 1-3 mM of calcium, magnesium and sulphate, is always adequate. The required micronutrients include I, B, Mn, Zn, Mo, Cu, Co and Fe.

(B) Carbon Source:

Glucose, fructose, maltose or sucrose (2-4%) can be used as source of energy or carbon but sucrose is the preferred source for most of the cultures. The sucrose in the medium is rapidly converted into glucose and fructose. The glucose is absorbed first followed by fructose.

(C) Vitamins and amino acids:

Thiamine, pyridoxine and nicotinic acid are commonly used as vitamins in B_5 and MS media. The former is required for most cultures while latter two promote cell growth. Amino acids and organic supplements -Amino acids serve as source of reduced nitrogen. In case of inadequate nitrogen, complex organic nitrogen supplement like casein hydrolysate (0.1-1 g/l) may be supplemented. Glycine is commonly used amino acid. Other organic supplements are coconut milk, yeast extract, peptone and malt extract. However, synthetic media are preferred and organic supplement of unknown chemical nature is used only when it is essential.

(D) Plant Growth Regulators (PGR):

A balanced combination of PGR is required for sustained growth. Two types of combinations are used; one for cell proliferation consisting of (preferably) 2, 4-dichlorophenoxy acetic acid (2, 4-D) or 1-naphthalene acetic acid (NAA) and a cytokinin (kinetin, benzyl adenosine, 2-isopentyladencisine, zeatin, thidiazuron), another for regeneration essentially containing low auxin [(NAA, IAA, Indole butyric acid (IBA)] and a cytokinin in high amount, but not 2, 4-D as an auxin. 2, 4-D is known to induce cell proliferation but suppresses differentiation in dicot plants. However, 2, 4-D and 2, 4, 5-T (2, 4, 5-trichlorophenoxy acetic acid) are effective in inducing somatic embryogenesis in cereal (monocots) and herbaceous dicot cultures.

Chemical	Formula	Concentration
Macronutrients (10 X)		100 mL/L
Ammonium nitrate	NH_4NO_3	16.5
Potassium nitrate	KNO_3	19.0
Calcium chloride	$CaCl_2.2H_2O$	4.4
Magnesium sulfate	$MgSO_4.7H_2O$	3.7
Potassium dihydrogen orthophosphate	KH_2PO_4	1.7
Micronutrients (100 X)		10 mL/L
Manganese sulphate	$MnSO_4.4H_2O$	2.23
Zinc sulphate	$ZnSO_4.7H_2O$	0.86
Potassium iodide	KI	0.086
Cupric sulphate	$CuSO_4.5H_2O$	0.0026
Sodium molybdate	$Na_2MoO_4.2H_2O$	0.025
Cobalt (ous) chloride	$CoCl_2.6H_2O$	0.0026
Boric acid	H_3BO_3	0.62
Vitamin source (100 X)		10 mL/L
Nicotinic acid	$C_6H_5NO_2$	0.05
Thiamine hydrochloride	$C_{12}H_{17}ClN_4OS.HCl$	0.01
Pyridoxine hydrochloride	$C_8H_{12}N_2O_2.2HCl$	0.05
Glycine	$C_6H_{12}O_6$	0.2
Iron source (100 X)		10 mL/L
Sodium EDTA	$C_{10}H_{14}N_2O_8Na_2H_2O$	2.78
Ferrous sulphate	$FeSO_4.7H_2O$	3.72
Myo-inositol		0.1 g (freshly add)
Sucrose	$C_3H_5NO_3$	30 g
Phytagel		2 g

(Fig.14) MS media composition

Medium Preparation:

Medium is prepared by suitably diluting the appropriate amount of stock solutions for desired volume of the medium. It may be advantageous to have separate stock solutions of calcium salt and potassium iodide.

All the ingredients are mixed, sugar added and pH is adjusted to 5.8-6.0 and medium is poured in the culture vessels. All the vessels are plugged with non-absorbent cotton, covered with aluminum foil and autoclaved at 121 °C for 15 min.

Prepared media can be stored for a few weeks before inoculation. Liquid medium for a given material is same as static medium used for callus cultures except gelling agent- agar. All the ingredients should be thoroughly mixed before dispensing in the vessels.

The following steps outline the proper preparation of media for tissue culture:

1. Mix a M.S. medium with the appropriate amount (500ml) of distilled water.
2. Now mix well the M.S. media and put on the hot plate for proper mixing.
3. Set the PH at 5.6-5.8.
4. Add agar to the beaker (12g).
5. Add hormone (if using).
6. Add anti-bacterial (Streptomycin 0.2%) and anti-fungal (Bavistin 0.4%) to prevent bacterial and fungal contamination respectively with appropriate amount.
7. Autoclave media for sterilization.
8. Dispense the melting media into petriplate or magenta box and make sure that each petriplate or magenta box is labeled.
9. The media should be left in a sterile environment, where it is monitored until it can be used.

3.4. Sterilization

It is a normal practice by which we can make anything pathogen/microbes free.

a. Sterilization of glassware:

We can use disposable glassware or use hot air oven/ microwave oven for sterilization.

b. Sterilization of media:

Three types of sterilization are used:

1. Dry heat
2. Wet heat
3. Filter sterilization.

1. Dry heat:

Glassware, metal tools and other articles, which do not get charred by high temperature, are put in containers or wrapped in paper or thick aluminum foil and placed in dry oven and sterilized for a period of not less than three hours at a temperature of 140-160 °C. Media and plastic ware cannot be sterilized by this method.

2. Wet heat:

The most popular method of sterilization both equipment and media is autoclaving at 121°C with a pressure of 15 psi (pounds per square inch) for 15 min (1.02 kg/cm^2). Modern autoclaves are capable of providing saturated steam treatment ranging from 70-132 °C, which is a pressure of up to 25 psi. All the vessels containing medium should be placed vertically and should not be filled more than 40% to their total capacity.

3. Filter sterilization:

Filter sterilization or cold sterilization is used when a solution or medium cannot be sterilized by autoclaving. It is the property of the filter (porosity 0.22 to 0.45 µm) to retain the

entire microorganism and make the solution free from microbes. This exclusion of microorganisms makes the solution sterilized without heating or autoclaving.

There are a variety of wet and dry heat treatments, radiations, filtration and gas and chemical treatments available for direct sterilization of material. Gas treatments are rarely used in the laboratory. Ultraviolet light treatment of working surfaces and sterile rooms are used in the labs while gamma radiation is used in the industry for the preparation of pre-sterilized disposable plastic-wares.

c. Sterilization of tools:

Surgical blades and scalpels are not sterilized by dry heat because the high temperature makes the cutting edge dull. Such articles including spatula and forceps are usually immersed in 70% v/v (volume by volume) ethyl alcohol until required, and sterilized during use by frequent immersion in alcohol and flaming.

d. Sterilization of explants:

1. *Seed sterilization is done by mercuric chloride and then rinse with distilled* water.

2. Leaf sterilization is done by absolute alcohol and then rinse with distilled water.

3. Inflorescence/Pollen grains sterilized is done by UV rays.

The explants chosen by us sterilized by following methods:

- Firstly explant washed by tap water (2-3times) and then with liquid detergent and then again wash with tap water.

- Use mercuric chloride (0.5g) for 30 seconds and then rinse with distilled water.

- Now just dip explant in 70% alcohol for milliseconds.
- Then again rinsed explant with distilled water
- Now explant is ready to inoculate in MS media (Fig.15).

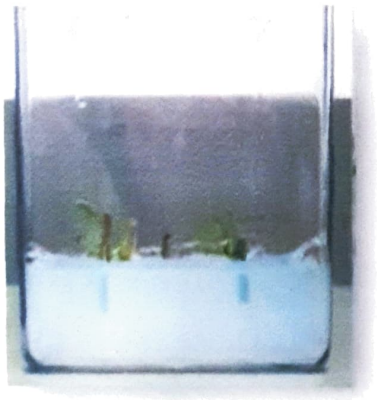
e. Sterilization of culture room:

Culture room is sterilized by UV rays before using.

Following care should be taken:

1. Minimize the air current in the working area so it is possible to avoid spores of contaminating microorganisms to move in along with the air currents over the sterile areas. At least, fan should not be used in laminar air flow bench room (inoculation room). Preferably, all the places should be air-conditioned.
2. Store properly the prepared media, nutrients and tools in cabinets.
3. Use separate area for cleaning and washing and for the preparation of medium.

UV tubes are also fixed in Laminar air flow bench placed in inoculation chamber. All these UV tube lights should be used frequently before inoculations. UV lights of corridors may be left open during nights.

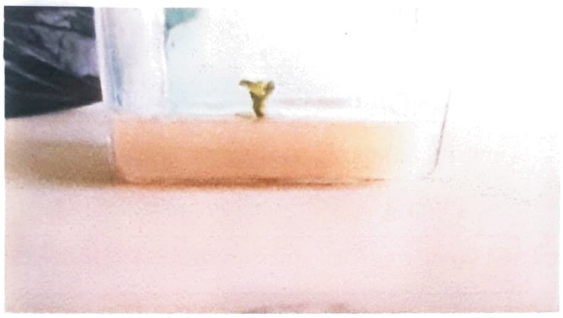


(Fig.15) Explant inoculated in MS media.

CHAPTER 4

RESULT

Firstly we prepared MS media. Then we select explant from ground of our college. And explant was sterilized by Mercuric chloride and 70% ethanol, then washed with distilled water. The explant was ready to inoculate in media (Fig.16)



(Fig.16)

After 14 days of inoculation of explant. The shoots arised from the nodal portion of *Tagetes erecta (*Fig.17)

(Fig.17) Shoot arise from nodal portion

After some days of sub culturing, explant became dead due to fungal contamination.

CHAPTER 5

CONCLUSION

Marigold botanically identifies as *Tagetes* (Compositae) genus is an ethno botanically known drug, used from ancient times in the Indian system of Medicine for the treatment of rheumatism, cold, bronchitis, eye diseases, ulcers etc. *Tagetes* species, commonly known as marigold, are grown as ornamental plants and thrive in varied agro-climates. The genus has been recognized as a potential source of very interesting biologically active products i.e. carotenoids that are used as food colorants, feed additives and possess anticancer and antiageing effects, essential oil known for antimicrobial and insecticidal properties, thiophenes with a marked biocidal activity and flavonoids having pharmacological properties. The *Tagetes* oil has been mainly used for the compounding of high-grade perfumes and also acts as antihaemorrhagic, anti-inflammatory, antiseptic, antispasmodic, astringent, diaphoretic and emmenagogue. This genus has been investigated for various biological activities like antimicrobial, antiplasmodial, antioxidant, insecticidal etc. The present review summarizes the biological activities and phytoconstituents of this genus.

Micro propagation (Fig.18) is the artificial process of producing plants vegetatively through tissue culture or cell culture techniques. In this artificial process of propagation, plants are produced invitro by asexual means of reproduction or by vegetative propagation.

Stage 1: Establishment of Aseptic Culture.
Stage 2: Multiplication of Explants.
Stage 3: Rooting of Regenerated Shoots or Somatic Embryo Germination.
Stage 4: Acclimatization or Transferring of Plantlets to Soil.

To grow the *Tagetes erecta* artificially, different instruments and tools are used like laminar air flow, autoclave for sterilization of explant and media and glass wares. Different glass wares like beaker, pipette, petriplate and glass rod. Different tools like magenta box, forceps, scalpel, burner etc.

Murasshige and Skoog media, or MS media, is the most widely-used plant culture medium and is available as basal salt mixtures or media containing organics. Regardless of format, MS media is comprised of micro- and macronutrients, plus nutrients like sugar, vitamins, and growth regulators.

The explant is *Tagetes erecta* whose nodal portion is selected for micro propagation. Explant is sterilized by different methods (70% ethanol, mercuric chloride) and washed with distilled water. Now explant is ready to inoculate into MS media and after some days, we get multiple shoots from nodal portion. After 14 days, the explant is sub cultured (transferred to fresh media).

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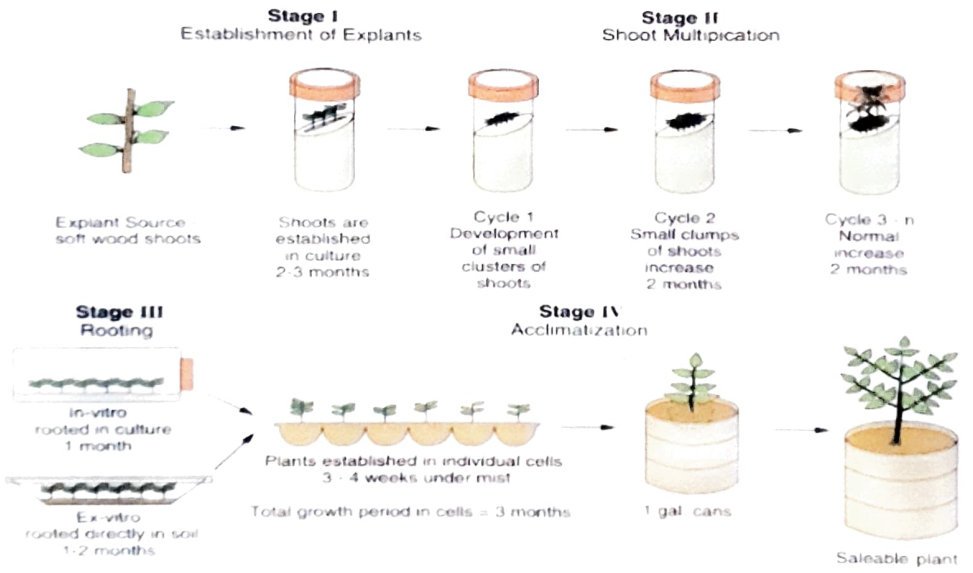

(Fig.18) Stages of micro propagation

- After some days of subculturing, the media get contamination by fungus and plant unable to grow in this condition.

CHAPTER 6

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